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Five-Year Longitudinal Assessment (2008 to 2012) of E-101 Solution Activity against Clinical Target and Antimicrobial-Resistant Pathogens

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This study summarizes the topical E-101 solution susceptibility testing results for 760 Gram-positive and Gram-negative target pathogens collected from 75 U.S. sites between 2008 and 2012 and 103 ESKAPE pathogens. E-101 solution maintained potent activity against all bacterial species studied for each year tested, with MICs ranging from <0.008 to 0.25 μg porcine myeloperoxidase (pMPO)/ml. These results confirm that E-101 solution retains its potent broad-spectrum activity against U.S. clinical isolates and organisms with challenging resistance phenotypes.

E-101 solution is a novel antimicrobial agent with broad-spectrum activity against both Gram-negative and Gram-positive pathogens, including drug-resistant strains. E-101 solution is an enzyme-based topical formulation composed of glucose oxidase (GO) and porcine myeloperoxidase (pMPO) in an aqueous vehicle. Topical application of E-101 solution with glucose activates *in situ* GO production of hydrogen peroxide (H_2O_2) that drives pMPO-dependent oxidation of chloride to hypochlorous acid (HOCl). The reaction of H_2O_2 with HOCl yields singlet molecular oxygen ($^1O_2$). The microbicidal action of E-101 solution involves the binding of pMPO to the surfaces of target microorganisms, where HOCl and $^1O_2$ effect direct oxidative damage to the microorganisms (1–6).

E-101 solution represents the first-in-class topical myeloperoxidase-based microbicidal formulation applied directly to surgical wounds to prevent surgical-site infections (SSI). It is currently being tested in a pivotal phase 3 clinical trial (i.e., the Triple-IN Study) for the prevention of SSI after colorectal surgery. Infections caused by antimicrobial-resistant pathogens have become common and are increasingly difficult to treat (7). As part of the clinical development of E-101 solution, it is important to monitor any changes in activity that may indicate the emergence of resistance. This study reports the *in vitro* susceptibility results of E-101 solution against target SSI pathogens isolated from relevant clinical specimens from patients seeking medical care in the United States over a 5-year period (2008 to 2012). In addition, we evaluated the activity of E-101 solution against highly resistant ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens and other current problem pathogens with emerging resistance (8, 9).

A total of 760 nonduplicate, nonconsecutive clinical isolates of *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*), and *Pseudomonas aeruginosa* collected from 75 sites across the 9 U.S. census regions were evaluated (Table 1). Among the isolates tested were those with multidrug-resistant (MDR) phenotypes. A total of 103 nonduplicate clinical isolates of ESKAPE pathogens were preselected based on resistance phenotype and genetic characterization from both the Eurofins Medinet and NARSA repositories (Table 2). Evaluated ESKAPE pathogens included the following: *S. aureus*, including 10 hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) isolates (1 USA600 and 9 USA100 clones), 10 community-acquired MRSA isolates (CA-MRSA) (10 USA300 clones), 7 daptomycin-nonsusceptible *S. aureus* isolates, 9 linezolid-resistant *S. aureus* isolates, and 8 vancomycin-nonsusceptible *S. aureus* isolates (3 vancomycin-resistant *S. aureus* [VRSA] and 5 vancomycin-intermediate *S. aureus* [VISA] isolates); *Enterococcus* spp., including 9 vancomycin-resistant (5 *E. faecalis* and 4 *E. faecium*) isolates; *Enterobacteriaceae*, including 10 extended-spectrum-β-lactamase (ESBL)-producing isolates (5 *E. coli* and 5 *K. pneumoniae* isolates), 10 carbapenemase-producing *K. pneumoniae* (KPC) isolates, and 10 derepressed AmpC cephalosporinase-producing isolates (5 *Citrobacter* spp. and 5 *Enterobacter* spp.); and 20 MDR isolates, including 10 *Acinetobacter baumannii* and 10 *P. aeruginosa* isolates. ESBLs were phenotypically defined on prior testing as a >2-fold reduction in ceftazidime/cefotaxime MIC when combined with clavulanic acid (10). KPC-2 and KPC-3 carbapenemases were confirmed by real-time PCR (11). Derepressed AmpC cephalosporinase was phenotypically defined on prior testing as resistant to cefoxitin, ceftazidime, and cefotaxime, not inhibited by clavulanic acid, and susceptible to carbapenems (12). MDR bacteria were classified based on prior testing and on recommended guidelines (13). MDR bacteria were defined as nonsusceptible to ≥1 antimicrobial agents in ≥3 antimicrobial classes.

Antimicrobial susceptibility testing was performed using a modified broth microdilution method based on CLSI M7 guidelines as previously described (14, 15). Modifications to CLSI M7 were made to accommodate the rapid *in vitro* activity of E-101 solution. In the modified method, the first E-101 enzyme solution containing pMPO was diluted in double-strength cation-adjusted Mueller-Hinton broth (CAMHB), resulting in doubling dilutions throughout the microdilution panel. Next, a standardized bacterial suspension, prepared in double-strength glucose substrate solution, was added to each well and mixed with enzyme

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TABLE 1 Activity of E-101 solution against target bacterial pathogens[a]

Organism	2008–2010				2011				2012			
	No. of isolates tested	MIC (μg/ml)			No. of isolates tested	MIC (μg/ml)			No. of isolates tested	MIC (μg/ml)		
		Range	50%	90%		Range	50%	90%		Range	50%	90%
S. aureus	50	≤0.008–0.03	0.015	0.015	50	≤0.008–0.015	0.015	0.015	50	≤0.008–0.015	≤0.008	≤0.008
CoNS[b]	30	≤0.008–0.03	0.015	0.015	30	≤0.008–0.03	≤0.008	0.015	30	≤0.008->0.03	0.015	0.03
E. faecalis	15	0.06–0.25	0.25	0.25	15	0.12–0.25	0.25	0.25	15	0.06->0.5	0.25	0.25
E. faecium	15	0.03–0.12	0.06	0.12	15	0.06–0.25	0.06	0.12	15	0.03–0.25	0.06	0.12
E. coli	30	0.015–0.12	0.12	0.12	30	0.12–0.25	0.25	0.25	30	0.06–0.25	0.12	0.12
K. pneumoniae	30	0.06–0.25	0.25	0.25	30	0.06–0.25	0.12	0.25	30	0.03–0.25	0.12	0.12
Enterobacter spp.	30	0.03–0.12	0.12	0.12	30	0.06–0.25	0.12	0.12	30	0.03–0.12	0.06	0.06
P. mirabilis	30	≤0.008–0.06	0.03	0.03	30	0.015–0.06	0.03	0.06	30	0.015–0.06	0.03	0.03
P. aeruginosa	30	≤0.008–0.12	0.03	0.06	30	0.015–0.25	0.06	0.12	30	0.015–0.12	0.03	0.06

[a] MICs are the concentrations of pMPO (μg/ml) in E-101 solution.
[b] CNS, coagulase-negative staphylococci.

dilutions. Each antimicrobial agent and enzyme solution was diluted in CAMHB and dispensed in microdilution panels. Isolates were prepared by suspending several colonies from an overnight culture on Trypticase soy agar with 5% sheep blood in sterile saline, and the density was adjusted to a turbidity equivalent to that of a 0.5 McFarland standard (~10^8 CFU/ml). Standardized bacterial suspensions were further diluted in double-strength glucose substrate solution and mixed with serial drug dilutions to achieve a final concentration of 5×10^5 CFU/ml. E-101 solution begins to generate reactive oxygen species immediately postinoculation, The microdilution panels were incubated in ambient air at 35°C for 18 to 24 h. MICs were determined by observing the lowest concentration of antimicrobial agent that inhibited growth of the organisms and was expressed as μg pMPO/ml in E-101 solution. Quality control ranges determined for E-101 solution for *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were 0.01 to 0.03 μg pMPO/ml and 0.15 to 0.5 μg pMPO/ml, respectively (14). Isolates were concurrently tested against relevant comparators in accordance with CLSI M7 (13). Quality control organisms included *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853.

Table 1 summarizes the MICs of E-101 solution for each year tested. Based on MIC_{50}s and MIC_{90}s, E-101 solution maintained a high level of activity (± one doubling dilution). From 2008 to 2012, we observed no increase in the MIC distribution of isolates tested. The E-101 solution MIC ranges for the staphylococci maintained the highest level of potency (≤0.008 to 0.03 μg pMPO/ml). The distribution of MICs was very similar for all phe-

TABLE 2 Activity profile of E-101 solution against evaluated ESKAPE pathogens[a]

Organism	Phenotype	No. of isolates	MIC (μg pMPO/ml)			
			Range	Mode	50%	90%
S. aureus	Overall	44	≤0.008–0.06	≤0.008	≤0.008	0.015
	HA-MRSA[b]	10	≤0.008–0.015	≤0.008	≤0.008	≤0.008
	CA-MRSA[b]	10	≤0.008–0.015	0.015	0.015	0.015
	DAP NS	7	≤0.008–0.06	≤0.008	NA	NA
	LZD R	9	≤0.008–0.015	≤0.008	NA	NA
	VAN NS	8	≤0.008–≤0.008	≤0.008	≤0.008	≤0.008
Enterococcus spp.	VRE	9	0.03–0.12	0.12	NA	NA
Enterobacteriaceae	Overall	30	0.015–0.25	0.12	0.12	0.12
	ESBL[c]	10	0.06–0.25	0.12	0.12	0.12
	KPC[d]	10	0.12–0.12	0.12	0.12	0.12
	Derepressed AmpC[e]	10	0.015–0.06	0.06	0.06	0.06
A. baumannii	MDR[f]	10	0.03–0.03	0.03	0.03	0.03
P. aeruginosa	MDR[f]	10	0.015–0.06	0.03	0.03	0.06

[a] HA-MRSA, hospital-acquired methicillin-resistant *S. aureus*; CA-MRSA, community-acquired MRSA; DAP NS, daptomycin nonsusceptible; LZD R, linezolid resistant; VAN NS, vancomycin nonsusceptible; VRE, vancomycin-resistant enterococci; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; AmpC cephalosporinase; MDR, multidrug resistant.
[b] HA- and CA-MRSA designations are based on the USA type reported in the NARSA (Network on Antimicrobial Resistance in *Staphylococcus aureus*) repository.
[c] Phenotypically positive in prior testing, with a >2-fold reduction in ceftazidime/cefotaxime MIC when combined with clavulanic acid (10).
[d] Positive for KPC-2/KPC-3 by PCR (11).
[e] Phenotypically positive based on prior testing (cefoxitin resistant, ceftazidime resistant, cefotaxime resistant, not inhibited by clavulanic acid, carbapenem susceptible) (12).
[f] MDR was based on prior testing and defined as resistance to 3 or more different antimicrobial classes (13).

notypes tested. The E-101 solution MIC ranges for *E. faecalis* and *E. faecium* were very similar during each test period. E-101 solution was consistently more active against *E. faecium* (MIC_{50}, 0.06 μg pMPO/ml, and MIC_{90}, 0.12 μg pMPO/ml) than *E. faecalis* (MIC_{50} and MIC_{90}, 0.25 μg pMPO/ml). The E-101 solution MIC_{50}s and MIC_{90}s obtained for each species among the *Enterobacteriaceae* also reflected a high level of potency of E-101 solution that has been maintained against most recent organisms. Multidrug resistance among *Enterobacteriaceae* evaluated had no impact on E-101 solution activity. The E-101 solution MIC_{50}s and MIC_{90}s for *P. aeruginosa* also showed a high level of potency of E-101 solution that has been maintained against most recent organisms, including MDR strains.

The activity of E-101 solution against resistant ESKAPE pathogens was equivalent to that observed for E-101 solution during recent surveillance testing, where isolates with these phenotypes were infrequent or not encountered (Table 2). E-101 solution maintained potent MICs (0.015 μg pMPO/ml or less excluding one daptomycin-nonsusceptible isolate) overall against *S. aureus* isolates, including those resistant to current agents against Gram-positive organisms. E-101 solution was active against prevalent HA-MRSA and CA-MRSA clones. Among the enterococci, E-101 solution maintained potency against vancomycin-resistant enterococci, with MICs in the 0.03- to 0.12-μg pMPO/ml range. E-101 solution MICs were 2-fold lower against vancomycin-resistant *E. faecium* (0.03 to 0.06 μg pMPO/ml) than vancomycin-resistant *E. faecalis* (0.06 to 0.12 μg pMPO/ml). Against various species and types of β-lactamase-producing *Enterobacteriaceae*, E-101 solution maintained potent MICs, including the recently emerged KPC-producing *K. pneumoniae*. Against *A. baumannii*, including all evaluated MDR *A. baumannii* isolates, E-101solution had MICs of 0.03 μg pMPO/ml. E-101 solution was also highly active against MDR *P. aeruginosa*, with an MIC_{50} of 0.03 μg pMPO/ml and an MIC_{90} of 0.06 μg pMPO/ml.

Prevention of SSIs remains an important medical need in high-risk surgical patient populations, such as colorectal surgery patients, transplant patients, and patients receiving implant devices. Improvement in infection control practices and topical antiseptics that are tissue safe and broadly microbicidal for any potential pathogen for wound management are needed. Topical antiseptics currently on the market are associated with local tissue injury and impairment of wound healing when applied directly to clean or infected wounds. E-101 solution is intended for direct application to wounds and wound margins without associated tissue injury. Previous studies using the partial full-thickness pig wound model have shown that topical application of E-101 solution to surgical wounds does not impair subsequent wound healing (16). Furthermore, E-101 solution has demonstrated *in vivo* efficacy in whole-animal models of surgical infection prevention (17, 18).

Across *in vitro* studies to date, E-101 solution has demonstrated greater overall *in vitro* activity than gentamicin and mupirocin against both Gram-negative and Gram-positive pathogens (14). Data from this surveillance study confirm earlier studies and demonstrate that E-101 solution had high *in vitro* potency and a broad spectrum of activity against many circulating isolates in the United States during 2008 and 2012. In addition, E-101 solution was potent *in vitro* against ESKAPE pathogens, which constitute clinically important pathogens with problematic mechanisms of resistance to commonly utilized agents. The emergence and spread of resistance combined with the increasing prevalence of multidrug resistance among ESKAPE pathogens have left relatively few effective therapeutic options for the treatment of drug-resistant infections. This attribute highlights the important utility of E-101 solution as a prophylactic agent for the prevention of SSIs, where resistant organisms are likely to be encountered, as well as other superficial infections caused by resistant organisms.

In summary, the MIC results of this longitudinal 5-year surveillance study indicate that E-101 continues to maintain a high level of activity against all target organism groups. No significant longitudinal changes in MICs further support the efficacy of E-101 solution, especially for preventing SSIs with organisms likely to be encountered clinically at present, including those with problematic resistance to other currently utilized agents. Continued monitoring of E-101 solution activity is warranted during clinical testing so that any changes in the level of activity can be detected in a timely matter.

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